Production and Characterization of Bioactive Molecule of Microbial Origin against Multi Drug Resistant strains of *Pseudomonas aeruginosa*

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Production and Characterization of Bioactive Molecule of Microbial Origin against Multi Drug Resistant strains of *Pseudomonas aeruginosa*

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Abstract:

Pseudomonas aeruginosa is gram-negative and opportunistic pathogen associated with nosocomial infections. Bacteremia is life-threatening in the immunocompromised host. Increasing frequency of multi-drug-resistant (MDR) PA strains is concern with the membrane permeability, efflux pump, enzymatic degradation, target site alteration. Antibiotic resistance is occurring in almost all classes of antibiotic Therefore, the present study is about to search an effective and novel bioactive compound from microbial isolates which will be isolated from extreme environment and its bioactivity will be determined against MDR *Pseudomonas aeruginosa*. The optimization studies during production of secondary metabolites will enhance qualitative and quantative production of secondary metabolites, the potent secondary metabolites will be characterized structurally and for its spectrum of activity and target sites in *Ps. aeruginosa* will be analysed, the microbial isolates having abilities in production of lead compound will be identified using molecular biology tool

Keywords: *Pseudomonas aeruginosa*, secondary metabolites, extremophilic bacterial and fungal isolate

Introduction:

Infectious diseases have a large impact on public health. Disease can occur at any body site and can be caused by the organism itself or by the body's response to its presence. Bacteria are transmitted to humans through air, water, food, or living vectors. The principal modes of transmission of bacterial infection are contact, airborne, droplet, vectors, and vehicular. Preventive measures have a dramatic impact on morbidity and mortality. Such measures include water treatment, immunization of animals and humans, personal hygiene measures. Bacterial resistance to antibiotics is a growing concern mandating their prudent use.

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, aerobic rod-shaped bacterium that can cause disease in plants and animals, including humans. Of the many different types of *Pseudomonas*, the one that most often causes infections in humans is *Ps. aeruginosa*

which can cause infections in the blood, lungs pneumonia (Barbier et al., 2013), meningitis, abscess, soft tissue infections, urinary tract infections, catheter associated infections, corneal infections and conjunctival erythema. (Chatterjee et al, 2016) and also other parts of the body after surgery

Ps. aeruginosa is the one of the most common causative agents of pneumonia in patients with HIV and organ-transplant recipients (Afessa and Green, 2000) and the rate of *Ps. aeruginosa* infections in HIV patients is on rise (Swathirajan et al., 2019). *Ps. aeruginosa* has become an emerging opportunistic pathogen in the clinics. Recent epidemiological studies demonstrate its nosocomial pathogen status, particularly those strains with increased antibiotic resistance *Ps. aeruginosa* exploits weaknesses in host defense to mount an infection. Indeed, *Ps. aeruginosa* is the epitome of an opportunistic pathogen of humans.

The bacterium hardly infects uncompromised tissues, but it can invade any tissue beleaguered by immunodeficiency. *Ps. aeruginosa* causes infection in the urinary tract, respiratory system, dermis, soft tissue, bacteraemia, bone and joint, gastrointestine and blood, particularly in patients with severe burns, tuberculosis, cancer and AIDS. Importantly, *Ps. aeruginosa* causes a significant problem in patients hospitalized with cancer, cystic fibrosis and burns, with 50% fatality (Hancock, 1998).

According to the Centers for Disease Control and Prevention (CDC), the overall incidence of *Ps. aeruginosa* infections in US hospitals averages about 0.4% (4 per 1000 discharges), and the bacterium is the fourth most common isolated nosocomial pathogen, accounting for 10% of all hospital-acquired infections.

Ps. aeruginosa has been shown to form biofilm-like microcolonies in the lungs of cystic fibrosis (CF) patients (Bjarnsholt et al., 2009). Singh et al., 2000 found that quorum sensing production signals found in patients lungs were only produced by isolated strains when they were grown in biofilms. These two lines of evidence suggested that *Ps. aeruginosa* forms biofilms in the CF lung, possibly explaining the difficulty of treating this infection.

The colistin, tobramycin and gentamicin are currently the most commonly prescribed nebulized antibiotics. (Matuschek et al.,2018) Much effort is directed at treating chronic *Ps. aeruginosa* infection but as chronic infection is seldom if ever eradicated when first established, prevention is preferable. Pseudomonal infections are increasingly resistant to certain antibiotics, and the organism may acquire resistance during therapy. Two agents from different classes should be used when the risk of antibiotic resistance is high (eg, in severe sepsis, septicemia, and inpatient neutropenia).

Pseudomonas infection can be treated with a combination of an antipseudomonal beta-lactam (eg, penicillin or cephalosporin) and an aminoglycoside. Carbapenems (eg, imipenem,

meropenem) with antipseudomonal quinolones may be used in conjunction with an aminoglycoside. The type of resistance observed in *Ps. aeruginosa* against Carbapenems (Buehrle et al., 2017), β-lactams, aminoglycosides, Fluoroquinolones, Colistin, and amino glycosides (Hancock and Speert, 2000) includes antibiotic inactivation, efflux systems membrane impermeability, porines and target modification. The standard antibiotic therapy may be inferior to some new comparator agents in the treatment of serious *Ps. aeruginosa* infections, especially in the setting of increased antimicrobial resistance.

Alternative approaches against MDR *Ps. aeruginosa* has been reported such as OligoG, peptidomometic antibiotics, bacteriophage, anti-biofilm and anti-QS statergis, gallium, immunotherapies (Wynne et al,2017) and cysteamines (Ferrari et al. 2017).Novel antibiotics with activity against *Ps. aeruginosa* are essential, the microorganism from extreme environment might have potential to produce secondary metabolites with bioactivity against multiple drug resistant *Ps. aeruginosa*. Hence in proposed research work the novel bacterial and fungal isolates will be isolated and screened against MDR *Ps. aeruginosa* and the bioactive compounds will be isolated and characterized

Review of literature:

Sekiguchi et al. (2007) has reported that *Ps. aeruginosa* is aerobic, non-spore forming, non-fermentative Gram negative bacillus, causes diseases to both community-acquired and hospital-acquired infections specially in hospitalized patients, immunocompromised hosts andprimary cause of morbidity and mortality in patients with cystic fibrosis.

Hancock and Quinn, (1998) has mentioned that *Ps. aeruginosa* is a frequent nosocomial pathogen and responsible for the infections in patients with cystic fibrosis; bronchiectasias; neutropenia; AIDS; and/or burn and patients with metabolic, hematologic, or malignant diseases. This bacterium is mainly responsible for ventilator-associated pneumonia, surgical site infection, urinary tract infection, and sepsis in intensive care unit patients.(Corona Nakamura et al.2001) Most of the strains are commonly resistant to many antibiotics including imipenem and meropenem; therefore, it is usually a problematic pathogen in therapy**(**Ndip et al.2005**).**Infections generally limited to skin and soft tissues. Contaminated swimming pools, whirlpools and hot-tubs are a recognized cause of folliculitis and are associated with urinary tract infection in females

Those most at risk include patients in hospitals, especially those on breathing machines (ventilators), with devices such as catheters and with wounds from surgery or burns *Ps. aeruginosa* lives in the environment and can be spread to people in healthcare settings when they are exposed to water or soil that is contaminated with these germs. Resistant

strains of the germ can also spread in healthcare settings from one person to another through contaminated hands, equipment, or surfaces. *Ps. aeruginosa* infections are generally treated with antibiotics. (Ali and Alhajhusain, 2009)

Empirical antibiotic therapy for suspected cases of *Ps. aeruginosa* includes mono therapy and combination therapy; this therapy reduces the mortality in patients with severe *Ps. aeruginosa* infections (Park et al., 2012). However, Lister et al., (2009) has reported treatment of *Ps. aeruginosa* infections has become a great challenge due to the ability of bacterium to resist many of the currently available antibiotics.

The World Health Organization (WHO) has recently listed carbapenem-resistant *Ps. aeruginosa* as one of three bacterial species in which there is a critical need for the development of new antibiotics to treat infections (Tacconelli et al., 2017). Moreover, Hirsch and Tam (2010) mentioned that the excessive use of antibiotics during treatment accelerates development of multidrug-resistant *Ps. aeruginosa* strains, leading to the ineffectiveness of the empirical antibiotic therapy against this microorganism. *Ps. aeruginosa* displays resistance to a variety of antibiotics, including aminoglycosides, quinolones and β-lactams . (Hancock and Speert, 2000)

New drugs were discovered and developed at a breathtaking pace. Such efforts led to dramatic advances in human health, as bioactive secondary metabolites or antimicrobials were used to treat an increasingly wide range of infections while allowing for the evolution of complex medical care. Antimicrobial resistance is a dangerous and constantly increasing problem, leading to an increase in health care costs, decreased treatment options, and a higher incidence of mortality due to bacterial and fungal infections worldwide. Global initiatives are calling for urgent action not only to develop new antimicrobial therapies but also decrease the contribution of humans to increasing antimicrobial resistance (World Health Organization, 2015).

Amorim et al (2020) has concluded the excessive and inappropriate use of antibiotics continues to be a major factor in the expansion of antimicrobial resistance, and the search for new drugs has been an alternative to minimize the damage caused by this phenomenon

Cigana et al., (2016) has reported development of new antibiotics or alternative therapeutic strategies for treatment of *Ps. aeruginosa* infections is urgently required for the patients whose infections are resistant to conventional antibiotics. New antibiotics with novel modes of action have been explored in recent years, as have new routes of administration and resistance to modification by bacterial enzymes (Walkty et al., 2014). Some of these newer antibiotics show excellent in vitro antibacterial activity against *Ps. aeruginosa* as well as lower minimum inhibitory concentration (MIC) compared to conventional antibiotics (El

Solh and Alhajhusain, 2009).

In addition, recent studies have reported several novel non-antibiotic therapeutic approaches that are highly effective in killing antibiotic resistant *Ps. aeruginosa* strains. These approaches include: inhibition of quorum sensing and bacterial lectins, use of iron chelation, phage therapy, vaccine strategy, nanoparticles, antimicrobial peptides and electrochemical scaffolds. These therapeutic approaches can be used as either an alternative to or in combination with conventional antibiotic treatments (Chatterjee et al., 2016)

Zheng Pang et al, (2019) has stated that the eradication of *Ps. aeruginosa* has become increasingly difficult due to its remarkable capacity to resist antibiotics. Strains of *Pseudomonas aeruginosa* are known to utilize their high levels of intrinsic and acquired resistance mechanisms to counter most antibiotics.

In addition, adaptive antibiotic resistance of *Ps. aeruginosa* is a recently characterized mechanism, which includes biofilm mediated resistance and formation of multidrug-tolerant persisted cells, and is responsible for recalcitrance and relapse of infections.

Objectives of Research:

- Isolation/procuring of MDR *Ps. aeruginosa* strains
- Determination of MIC of antibiotics against MDR *Ps. aeruginosa*
- Isolation and screening of bacterial and fungal isolates
- Production of bioactive compounds from isolated bacteria and fungi.
- Extraction and purification of bioactive compounds
- Bioassays of bioactive compound against MDR strains of *Ps. aeruginosa*
- Characterizations of bioactive compound
- Identification of target site of bioactive compound in MDR strains of *Ps. aeruginosa*.

Hypothesis:

The bacterial and fungal species from unique environment may have ability to produce secondary metabolite due to expression of unique genes under the stress condition. The expressed gene product might have potential to inhibit the growth of *Ps. aeruginosa.* This bioactive molecule will be effective for the controlling the infection caused by the *Ps. aeruginosa.*

Methodology to be adopted:

- MDR strain of *Ps. aeruginosa* will be isolated from clinical sample by using enrichment and selective media.
- Procuring of MDR strain of *Ps. aeruginosa*
- Determination of MIC of antibiotics against isolated and procured MDR stains of *Ps. aeruginosa*
- Isolation of bioactive molecule producing bacterial and fungal strains from unique ecological niches.
- Screening of isolated MDR strain of *Ps. aeruginosa* will be carried out against bioactive secondary metabolites of bacteria and fungi by bioassay methods
- Production of bioactive secondary metabolite will be carried out and purification of antipseudomonal compound will be done by using analytical methods.
- For the characterization of bioactive secondary metabolite UV Spectroscopy, IR spectroscopy, GC-MS, NMR, MALDI-TOF, will be used.
- The target site in MDR strain of *Ps. aeruginosa* will be identified.

Importance of study:

The novel lead bioactive compound will be isolated and characterized from microorganism against MDR *Ps. aeruginosa*. This compound will be more potent and with minimum adverse effect on human during treatment of *Ps. aeruginosa* infection or it can augment actions of anti-pseudomonas drug

National Status:

Against the severe problem of MDR *Ps. aeruginosa*, Ramachandran et al (2019) from Medical Microbiology & Marine Pharmacology Unit, Department of Marine Science, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India has obtained a noval bioactive compound from actinomycetal origin from marine environment and it has showed good activity and MIC of 120 µg/mL against MDR *P. aeruginosa, K. pneumoniae* and also related infectious diseases.

Beulah et al., (2021) from Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India has found the antibacterial activity of ethanol and water extracts of S. maritima leaves, the extracts has showed successful inhibiting the growth of tested bacteria but comparatively ethanol extract showed highest inhibitory zone against *Staphylococcus aureus (4.9±1.3), Bacillus subtilis (1.6±0.3), Klebsiella pneumoniae (4.2±1.8) and Ps. aeruginosa (4.1±1.2).*

Valli et al., (2012) from Research Department of Microbiology, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai has reported the effect of isolated actinomycetes sp C11 showed activity against *Staphylococcus species (13.0±0.5 mm), Vibrio harveyi (11.0±0.2 mm), and Pseudomonas species (12.0±0.3 mm). C12 showed activity against Staphylococcus species (16.0±0.4 mm), Bacillus subtilis (11.0±0.2 mm), Vibrio harveyi (9.0±0.1 mm), and Pseudomonas species (10.0±0.2 mm).*

Singh and Singh (2021) from Kanya Gurukul Campus, Gurukul Kangri Deemed to be University, Haridwar, India has found that the isolated *streptomyces sp* from, Ganga river and agricultural farm soil shows good activity against MDR strains of *Ps. aeruginosa* (7.3±0.33), *Enterobacter sp.*(22.3 ±0.33), *Bacillus subtilis* (16.3 ±0.88) and *Staphylococcus aureus* (7.6 ±0.33) uropathogens.

International Status:

Bodede et al (2022) from Westville campus, University of KwaZulu-Natal (UKZN), South Africa has studied the anti- *Pseudomonas aeruginosa* activity of a secondary metabolite dilactone terpenoid of C16 architecture was isolated from the fungal endophytes *Neofusicoccum luteum* of Kigelia Africana and Extracts from ZF 34, ZF 52 and ZF 91 of the fungi showed anti-*Ps. aeruginosa* activity, with ZF 52 presenting the best MIC of 19.53 µg/mL.

Mugabe et al. (2006) from Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Canada had found that the liposomal formulations deliver a sufficient amount of aminoglycosides into antibiotic impermeable *Ps. aeruginosa*

Significance of study:

The present study will be helpful in production of bioactive compound which will be effective against multidrug resistant strains *Ps. aeruginosa* with minimum side effects and toxicity to the patients or it can be also used in combination with other antibiotics which will prevent development of antibiotic resistance among, ***Ps. aeruginosa.***

Proposed work Plan:

Year-wise Plan of work and targets to be achieved:

Year	**Target to be completed**
First Year	❖ Coursework completion ❖ Review of literature &Sample collection ❖ Isolation of MDR strains of *Ps. aeruginosa*.
Second Year	❖ Isolation of bioactive compound producers microorganisms ❖ Identification of bioactive compound producers. ❖ Production and purification of Bioactive compounds
Third Year	❖ Screening of MDR *Ps. aeruginosa* against bioactive secondary metabolites ❖ Structural analysis of purified bioactive compounds
Fourth year	❖ Comparative study of bioactive compound in vitro. ❖ Target site interaction recongnization ❖ Resulting and data analysis ❖ Thesis writing and submission to University

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Research Student **Research Guide**

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